Pathogenicity of Stem-end Rot Associated Fungi Isolated from Karthakolomban Mango and Their Control by Spray and Fumigation Treatments with Selected Essential Oils

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ABSTRACT

Purpose: Stem-end rot (SER) is a major disease of mango that causes serious postharvest losses. Application of fungicides is environmentally unsound and is being practiced currently in its control. This study was conducted to develop essential oil treatment systems as eco-friendly strategies to control SER of Karthakolomban mango and to determine the pathogenicity of several SER associated fungi.

Research Method: Pathogenicity of four fungal isolates in SER was assessed by inoculating them on Karthakolomban mango fruits. Karthakolomban mango fruits were subjected to spray and fumigation treatments using four essential oils and their pathological, physicochemical and sensory properties were evaluated after a 8-day storage at 12 – 14 ºC.

Findings: Lasiodiplodia theobromae, Pestalotiopsis sp., Phomopsis sp. and Xylaria feejeensis were the major SER pathogens of mango. X. feejeensis was identified as a SER pathogen for the first time in Sri Lanka. Spray and fumigation treatments conducted using basil, clove, cinnamon leaf and cinnamon bark oils effectively controlled SER of Karthakolomban mango stored at 12 – 14 ºC for 8 days. Mango sprayed with 1.6 µL/mL cinnamon bark oil has displayed no SER after induced ripening. None of the treatments caused drastic alterations in physicochemical and sensory properties of mango.

Research Limitations: Storage period of mango was restricted to 8 days due to the initiation of natural ripening which was the major limitation to achieving a further enhanced shelf life.

Originality/Value: The treatment strategies developed by this research could be commercialized as bio-safe SER control strategies in reducing postharvest losses of mango in the local and international trade.

Keywords: Essential oils, fumigation, mango, pathogenicity, spray, stem-end rot

INTRODUCTION

Mango (Mangifera indica L.) is one of the most renowned tropical fruits consumed worldwide due to its delightful taste and higher contents of certain nutrients like vitamins A, B, C and fiber. Karthakolomban is one of the superior varieties of mango popular among growers as well as consumers in Sri Lanka due to its unique flavour and high nutritional value (Kothalawala and Jayasinghe, 2017). Highly perishable and delicate nature of mango fruits make them susceptible to postharvest damages, causing serious losses. About 20 – 25% of mango are wasted because of the inappropriate harvesting, packaging and storage practices (Singh et al., 2014). Any postharvest deterioration may result in mangoes with an undesirable quality and a shortened storage life, which will result in rejection of the fruit from the market (Alemu,
2014). Stem-end rot (SER) is one of the major diseases, which causes extreme postharvest losses of mango both in Sri Lanka and worldwide (Karunanayake et al., 2016). SER is a latent infection in which the pathogens remain dormant on mature mango fruit until they begin to ripe (Alemu, 2014). SER is caused by a group of pathogenic fungi, including Lasiodiplodia theobromae, Phomopsis mangiferae, Pestalotiopsis mangiferae, Dothiorella dominicana, D. mangiferae and Colletotrichum gloeosporioides worldwide (Abeywickrama, 2006; Alemu, 2014).

Indiscriminate use of fungicides on crops has been increased during the last few decades which could be detrimental to the environment and human health. Due to overuse of fungicides, a large amount of their residues accumulate in fruits and vegetables. The products with high amounts of fungicide residues are hazardous for human consumption (Muri et al., 2009). Such products become unsuitable for international market as they can exceed internationally accepted standards of Maximum Residue Levels (MRLs). In view of them, abiding by Good Agricultural Practices (GAP) and by shifting to Integrated Pest Management (IPM) strategies involving biological agents, botanicals is a timely need (Ehler, 2006; Gravani, 2009).

Exploitation of the essential oils of many higher plants to control postharvest deteriorations of fruits, has been accepted as a sustainable, efficacious, economical and eco-safe method by many scientists. The United States Food and Drug Administration (FDA) considers essential oils as Generally Recognized as Safe (GRAS) compounds (Nazzaro et al., 2017). Antimicrobial constituents of essential oils possess an array of modes of action against plant pathogens. Simple phenols present in essential oils cause membrane disruption of pathogens, while phenolic acids bind to adhesins which facilitate the adhesion to hosts, disrupt cell wall integrity and inactivate enzymes. Terpenoids cause membrane disruption and alkaloids can intercalate into cell wall. Tannins can bind with proteins and inhibit enzymes of pathogens. Flavonoids bind to adhesins, complex with cell walls and inactivate enzymes of pathogens. Coumarins interact with eukaryotic DNA which results in the inhibition of protein synthesis. Lectins and polypeptides can form disulfide bridges in proteins which alter the functions of enzymes of microorganisms (Zaker, 2016).

According to the preliminary investigations conducted at the Department of Botany, University of Kelaniya, Sri Lanka, in vitro liquid bioassay revealed the fungicidal efficacy of Ocimum basilicum (basil), Syzygium aromaticum (clove), Cinnamomum zeylanicum (cinnamon) leaf and bark oils against SER associated fungi of mango at 0.8, 1.0, 1.0 and 0.8 μL/mL concentrations, respectively. According to in vitro disc volatilization bioassay, the same oils were found to be fungicidal against SER pathogens at 16.0, 16.0, 8.0 and 4.0 μL/plate concentrations, respectively (Ekanayake, 2017; Kodituwakku, 2018).

Due to these verified antifungal properties, plant essential oils could be effectively used to control postharvest diseases of many fruits caused by different fungal pathogens. Therefore, the main objectives of the present study were (i) to examine the efficacy of spray and fumigation treatments with basil, clove, cinnamon leaf and cinnamon bark oils in controlling SER and enhancing shelf life of Karthakolomban mango stored at 12 – 14 ºC and (ii) to determine the pathogenicity of four fungal isolates (i.e. L. theobromae, Phomopsis sp., Pestalotiopsis sp. and X. feejeensis) previously isolated from Karthakolomban mango with SER.

MATERIALS AND METHODS

Mango fruits

90 days-old mature mango (cv. Karthakolomban) fruits green in colour with no record of pre-harvest fungicide treatment were obtained from orchards and home gardens in Gampaha District, Sri Lanka. All cut, bruised and pathologically damaged fruits were discarded.
and healthy, medium-sized fruits were selected for the experiments.

**Development of a disease severity index to monitor SER in Karthakolomban mango**

Four mango (cv. Karthakolomban) fruits were washed with running tap water to remove dirt, debris followed by sterile distilled water. Clean fruits were allowed to drip dry for 30 minutes on a laboratory bench, then placed in a plastic tray at room temperature. One selected fruit showing a gradual development of SER was photographed daily. Diseased area of the fruit in each photograph was estimated by DIGIMIZER (Version 5.3.4) and the disease severity was determined as percentage SER with respect to the total area of the fruit. A disease severity index was prepared using the photographs along with their percentage SER values (Fig.01).

**Pathogenicity assessment of previously isolated fungi from Karthakolomban mango with SER**

90 days old, mature and healthy mango (cv. Karthakolomban) fruits were washed with running tap water to remove dirt, debris and then in sodium hypochlorite (0.1% w/v) solution for surface sterilization. Subsequently, fruits were washed with sterile distilled water and allowed to drip dry for 30 minutes on a laboratory bench. Fruits were wounded on four sides at stem end to a depth of 1.5 mm by puncturing using a sterile pin. Each wound site was inoculated with a 7 mm diameter mycelial plug from the periphery of 7 days old pure cultures of *L. theobromae*, *Pestalotiopsis* sp., *Phomopsis* sp. and *X. feejeensis*. These fungi were previously isolated from Karthakolomban mango with SER and identity confirmed using morphological and molecular tools by Ekanayake *et al.*, (2019). Four sets of fruits were inoculated separately with each fungal isolate to test the individual contribution of each fungus towards SER disease and another set of fruits were inoculated with all fungal isolates together to test the collective contribution of the isolates towards the disease. A set of fruits inoculated with fresh PDA plugs served as the control. Each set of fruits consisting of five replicates were placed in plastic trays disinfected with ethanol (70% v/v). A piece of sterile cotton wool soaked in sterile distilled water was placed on each tray to provide moisture for fungal growth. Trays were covered with a polyvinylidene chloride (PVDC) film (Quanzhou Dealing Trading CO., LTD., China) and incubated at room temperature (28 ± 2 ºC) for 7 days (Tripathi and Shukla, 2009). Disease severity of each fruit was determined as percentage SER with reference to the total area of the fruit by tracing the diseased and total area onto a graph paper (Karunanayake, 2008). Further, disease severity was visually estimated by comparing with the developed SER disease severity index for Karthakolomban mango. The experiment was repeated once under identical conditions.

**Preparation of mango fruits for essential oil treatments**

Each mango fruit was washed with running tap water to remove dirt and debris and then in 1% (w/v) alum (potassium aluminum sulphate) (Devi Trading Company, Colombo 11, Sri Lanka) solution to remove latex. All fruits were washed with sterile distilled water and allowed to drip dry for 30 minutes on a laboratory bench. Fruits were wounded on four sides at stem end to a depth of 1.5 mm by puncturing using a sterile pin. Each wound site was inoculated with a 7 mm diameter mycelial plug from the periphery of 7 days old pure cultures of *L. theobromae*, *Pestalotiopsis* sp., *Phomopsis* sp. and *X. feejeensis*. These fungi were previously isolated from Karthakolomban mango with SER and identity confirmed using morphological and molecular tools by Ekanayake *et al.*, (2019). Four sets of fruits were inoculated separately with each fungal isolate to test the individual contribution of each fungus towards SER disease and another set of fruits were inoculated with all fungal isolates together to test the collective contribution of the isolates towards the disease. A set of fruits inoculated with fresh PDA plugs served as the control. Each set of fruits consisting of five replicates were placed in plastic trays disinfected with ethanol (70% v/v). A piece of sterile cotton wool soaked in sterile distilled water was placed on each tray to provide moisture for fungal growth. Trays were covered with a polyvinylidene chloride (PVDC) film (Quanzhou Dealing Trading CO., LTD., China) and incubated at room temperature (28 ± 2 ºC) for 7 days (Tripathi and Shukla, 2009). Disease severity of each fruit was determined as percentage SER with reference to the total area of the fruit by tracing the diseased and total area onto a graph paper (Karunanayake, 2008). Further, disease severity was visually estimated by comparing with the developed SER disease severity index for Karthakolomban mango. The experiment was repeated once under identical conditions.

**Spray treatment for mango with essential oils**

Basil oil was purchased from Aromatica laboratories (Pvt.) Ltd, Colombo 11, Sri Lanka. Clove oil and cinnamon leaf and bark oils were purchased from Citro Essential Oils (Pvt.) Ltd., Mt. Lavinia, Sri Lanka. Emulsions of each test essential oil were prepared based on the minimum lethal concentration (MLC) of a particular oil determined from *in vitro* liquid bioassays conducted by Ekanayake (2017) and Kodituwakku (2018). Each oil was added to distilled water (100 mL) to prepare oil emulsions and a drop of Tween 80 was added as a surfactant. Each mixture was stirred using a magnetic stirrer at 1000 rpm (R and M marketing, UK) for 10 minutes to obtain an emulsion. Emulsions of basil oil (1.6 µL/mL), clove oil (2.0 µL/mL), cinnamon leaf oil (2.0 µL/mL) and cinnamon bark oil (1.6 µL/mL)
were prepared according to this method. Each emulsion was transferred to a plastic sprayer and mixed well by shaking before applying on fruits. Negative control was prepared following the same procedure using distilled water (100 mL) and a drop of Tween 80. Carbendazim (Hayleys Agriculture Holdings Ltd., Colombo 10, Sri Lanka) (0.1% w/v) solution was used as the positive control (Abeywickrama et al., 2009).

Prepared mango (cv. Karthakolomban) fruits were sprayed separately with the oil emulsions, positive control (0.1% w/v carbendazim) and negative control (distilled water) solutions. The excess solutions were allowed to drain. All treated and control samples were placed separately in ventilated corrugated 3-ply fiberboard boxes (65 × 35 × 18 cm³) lined with perforated Manila paper (60 µm). Each box comprised of six fruits. All treatments and controls were stored at 12 – 14 ºC in a Walk-in cold room (Iceman Technologies (Pvt.) Ltd., Wattala, Sri Lanka) with a relative humidity (RH) of 85 – 90% for 8 days (Abeywickrama et al., 2009). The experiment was repeated once under identical conditions.

**Fumigation treatment for mango with essential oils**

Mango fruits prepared for essential oil treatments (as described under preparation) were placed (6 fruits per box) in ventilated corrugated 3-ply fiberboard boxes (65 × 35 × 18 cm³) lined with perforated Manila paper (60 µm). A piece of wettex sponge (15 × 15 cm²) (W. N. J. Importers and Exporters Pvt. Ltd., Dehiwala, Sri Lanka) was attached to the underside of the lid of each box using double-sided sticky tapes and a volume of a test oil was added using a micropipette onto wettex sponge based on the MLC values obtained from in vitro disc volatilization bioassays conducted by Ekanayake (2017) and Kodituwakku (2018). The volumes of oil added were basil (142 µL), clove (142 µL), cinnamon leaf (57 µL) and cinnamon bark (28 µL). Distilled water (500 µL) and ethanol (500 µL) were used as the negative and positive controls, respectively. All boxes were closed with lids and stored in the cold room at 12 – 14 ºC for 8 days (Anthony, 2003). The experiment was repeated once under identical conditions.

**Ripening of mango**

After cold storage of 8 days, mango fruits were subjected to induced ripening at room temperature (28 ± 2 ºC). All fruits were taken out from the packages and placed inside a plastic bucket of 18 L capacity. Ethepone (2-chloroethyl phosphonic acid) solution was prepared by dissolving 1 mL of Ethepone (480 g/L) (Ester, Summer Field Chemicals Pvt. Ltd., Horana, Sri Lanka) in 1 L of distilled water. Ethepone solution (10 mL) was taken to a 50 mL beaker and placed inside each bucket and evolution of ethylene (C₂H₂) was facilitated by adding 1 M sodium hydroxide solution (0.25 mL) to the beaker. The contents were made airtight by placing lids and facilitating ripening for one day. Lids of the buckets were removed and the fruits were allowed to ripen further one day until the mango attained the fully ripe stage (the fully yellow stage) (Abeywickrama et al., 2009).

**Assessment of pathological properties**

SER disease severity of five randomly selected ripened mango fruits from each treatment was visually recorded as % SER using the SER disease severity index for mango developed at the Department of Botany, University of Kelaniya (Fig.01).

**Assessment of physicochemical properties**

Five randomly selected ripened mango fruits from each treatment were analyzed for physicochemical properties. Total soluble solids (TSS) (ºBrix) of the filtrates of fruit pulp were determined using a hand-held refractometer (ATC-1E, ATAGO Co. Ltd., Japan). Titratable acidity (TA) (% citric acid) was assessed by a titration of the filtrates with 0.1 M NaOH using phenolphthalein as the indicator. pH of the filtrates was measured using a portable pH meter (PC 510, EUTECH Instruments, Singapore). Firmness of the fruit pulp was measured using
Assessment of peel colour

Peel colour of five randomly selected mango fruits from each treatment was visually assessed using a peel colour index for mango (cv. Karthakolomban) developed at the Department of Botany, University of Kelaniya (1 = Fully green, 2 = Breaker, 3 = More green than yellow, 4 = More yellow than green, 5 = Fully yellow).

Assessment of sensory properties

Five randomly selected ripened mango fruits from each treatment were provided to a ten-member untrained sensory panel along with a questionnaire to evaluate flesh colour, aroma, texture, taste, flavour and overall acceptability. Each sensory parameter was scored as follows: excellent = 9 – 10, good = 6 – 8, fair = 4 – 5, poor = 1 – 3 (Siriwardana, 2016).

Statistical analysis

Data obtained for physicochemical properties were analyzed using One-way ANOVA and mean separation was done using Tukey’s multiple comparison test. Kruskal Wallis non-parametric test was used to analyze data with respect to pathological properties, sensory properties and peel colour. Results of the pathogenicity assessment carried out using the graph paper method and SER severity index were subjected to One-way ANOVA and Kruskal Wallis non-parametric test, respectively (Siriwardana, 2016).

RESULTS AND DISCUSSION

SER disease severity index for Karthakolomban mango

Disease severity indices for fresh produce are important for assessing the extent of damage caused by a particular disease in order to develop suitable control strategies. The SER disease severity index (Fig.01) prepared during this study was used to evaluate the percentage SER severity of mango subjected to pathogenicity assessment of SER associated fungi and samples treated with essential oils.

![Figure 01: The disease severity index prepared to monitor SER development in mango (cv. Karthakolomban).](image-url)
Assessment of pathogenicity of SER associated fungi

According to the pathogenicity assessment carried out using the graph paper method described by Karunanayake (2008) and the SER severity index developed in our laboratory, collective contribution of all fungal strains towards SER in mango (cv. Karthakolomban) was found to be greater than that of each individual pathogen. When considering the individual contribution of each fungus towards SER in mango, *L. theobromae*, *Pestalotiopsis* sp. and *X. feejeensis* displayed higher percentage SER values than *Phomopsis* sp. According to this observation, contribution of *Phomopsis* sp. towards SER in mango could be less than that of other fungi, nevertheless it was a potential SER pathogen of mango. Percentage SER value estimated for a particular pathogen using the graph paper method was more-or-less similar to the percentage SER value assigned for the same pathogen by comparing with the SER severity index. Therefore, the subjective estimation of SER disease severity using the developed index might be almost accurate as the objective determination of SER severity using the graph paper method. The statistical analyses of both methods showed that all inoculation results are significantly different from the control (p < 0.05) (Table 01). The above four fungi produced almost same symptoms in inoculated mango fruits, which were similar to the characteristic symptoms of SER in mango including initiation of rot as a dark brown soft decay at the stem-end that gradually spread through the fruit, with the appearance of white colour mycelia on the lesions with time and draining of a straw colour fluid from the stem-end of the fruit (Fig.02).

Table 01: Percentage SER values obtained for Karthakolomban mango inoculated with SER associated fungi.

<table>
<thead>
<tr>
<th>Fungal isolate</th>
<th>% SER*</th>
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<tr>
<td></td>
<td>Graph paper method¹</td>
</tr>
<tr>
<td><em>L. theobromae</em></td>
<td>69.42⁺ ± 1.55</td>
</tr>
<tr>
<td><em>Pestalotiopsis</em> sp.</td>
<td>59.10⁺ ± 1.68</td>
</tr>
<tr>
<td><em>Phomopsis</em> sp.</td>
<td>14.81⁺ ± 1.28</td>
</tr>
<tr>
<td><em>X. feejeensis</em></td>
<td>59.26⁺ ± 1.26</td>
</tr>
<tr>
<td>All four pathogens</td>
<td>69.94⁺ ± 1.19</td>
</tr>
<tr>
<td>Control</td>
<td>1.17⁺ ± 0.16</td>
</tr>
</tbody>
</table>

*Percentage Stem-End Rot; Each data point represents the mean of eight replicates ± standard error.
¹Means sharing a common letter(s) are not significantly different by Tukey’s pair-wise comparison test at p < 0.05.
²Means sharing a common letter(s) are not significantly different by Kruskal Wallis non-parametric test at p < 0.05.

Figure 02: SER development in Karthakolomban mango after 7 days from inoculation with test pathogens; (A) control, (B) *L. theobromae*, (C) *Pestalotiopsis* sp., (D) *Phomopsis* sp., (E) *X. feejeensis* and (F) combination of all 4 pathogens.
L. theobromae, Pestalotiopsis and Phomopsis have been identified and confirmed by many studies as the causative organisms of SER in mango. A pathogenicity assessment conducted by Karunanayake et al., (2014) in Sri Lanka, confirmed L. theobromae (syn. Botryodiplodia theobromae) as a SER causing fungal pathogen of four cultivars of mango including Karthakolomban. The same fungus was identified as a SER pathogen of mango in Pakistan and its pathogenicity was confirmed by Syed et al., (2014). Pathogenicity of L. theobromae, P. mangiferae and Pestalotiopsis sp. towards causing SER of mango in Australia was established by Johnson et al., (1992). Therefore, the present pathogenicity assessment is in accordance with literature while confirming the identity of L. theobromae, Pestalotiopsis sp. and Phomopsis sp. as potential SER causing pathogens of mango. These fungi endophytically survive on branches of mango trees. They colonize inflorescence tissues endophytically and subsequently establish on stem-ends of young fruits. Once fruits initiate ripening due to the biochemical changes taking place in fruits, pathogens are responsible in bringing above characteristic SER symptoms (Johnson et al., 1992; Alemu, 2014).

However, X. feejeensis has not been documented as a causative agent of SER in mango before. Esiegbuya et al., (2013) reported a dry rot of the fruit of Raphia hookeri palm in Nigeria caused by X. feejeensis. Therefore, to the best of the knowledge of the authors, this might be the first reported evidence on the identification of X. feejeensis as a potential SER pathogen of mango (cv. Karthakolomban) in Sri Lanka. Nodulisporium sp. isolated from Karthakolomban mango with SER by Ekanayake et al., (2019) is a fungus which has not previously been reported as a postharvest pathogen. Due to the low survival rate of Nodulisporium sp. in vitro, it was not tested for pathogenicity. According to the phylogenetic analysis carried out by Ekanayake et al., (2019) for six SER associated fungi isolated from Karthakolomban mango, isolate numbers MH005088.1 and MH005089.1 were found to be clustered with L. theobromae, Pestalotiopsis sp., X. feejeensis and Nodulisporium sp. respectively. Isolate number MH005086.1 and MH005087.1 were clustered with Phomopsis sp. All isolate numbers given above are the accession numbers of the ITS sequences of each fungal isolate deposited in the GenBank (https://www.ncbi.nlm.nih.gov/genbank/).

**Spray treatment for mango with essential oils**

Pathological properties: Negative control samples sprayed with distilled water displayed the highest SER severity after storage at 12 – 14 ºC for 8 days and after induced ripening. No SER was observed in mango fruits sprayed with 1.6 µL/mL cinnamon bark oil and 0.1% (w/v) carbendazim (Fig.03). Mango fruits sprayed with 1.6 µL/mL basil, 2.0 µL/mL clove and 2.0 µL/mL cinnamon leaf oils exhibited comparatively lower % SER values than the negative control (i.e. 0.0 – 1.6%) with respect to the negative control (i.e. 17.2%) at p < 0.05. According to Karunanayake et al., (2016), spraying Karthakolomban mango fruits with a plant defense elicitor named as Bion® (Acibenzolar-s-methyl) and maintaining them in moist chambers at 28 ºC has effectively controlled postharvest diseases of mango including anthracnose and SER up to 7 days. Although Karunanayake et al., (2016) discovered very useful findings with regard to an alternative to synthetic fungicides, those findings are somewhat difficult to directly compare with the present study due to the use of different test compounds (such as essential oils and elicitor compounds) during the two separate studies, treatment methods and storage conditions. According to Sefu et al., (2015), spraying mango (cv. Apple Mango) fruits with cinnamon leaf oil (i.e. 0.25 – 0.75 µL/mL) and ginger oil (i.e. 1.50 – 4.50 µL/mL) has delayed anthracnose disease up to 5 – 10 days. Even though this study is focused on controlling anthracnose, results could be allied with present research because both studies reveal the potential
of cinnamon leaf oil in controlling postharvest fungal pathogens of mango. Karunanayake et al., (2018) reported that cardamom oil at 0.70 µL/mL has significantly reduced SER of Karthakolomban mango during a dip treatment. This result is in agreement with present study as most essential oils which have antifungal properties could effectively control SER of Karthakolomban mango at low concentrations.

Figure 03: SER disease severity of Karthakolomban mango subjected to spray treatment, stored at 12 – 14°C for 8 days and after induced ripening. T1: 1.6 µL/mL basil oil, T2: 2.0 µL/mL clove oil, T3: 2.0 µL/mL cinnamon leaf oil, T4: 1.6 µL/mL cinnamon bark oil, T5: negative control and T6: 0.1% (w/v) carbendazim. Each data point represents the mean of ten replicates. Means sharing a common letter(s) are not significantly different by Kruskal Wallis non-parametric test at p < 0.05.

Figure 04: Appearance of Karthakolomban mango subjected to spray and fumigation treatments, stored at 12 – 14°C for 8 days and after induced ripening. (A) Negative control, (B) 1.6 µL/mL cinnamon bark oil and (C) 0.1% (w/v) carbendazim treated mango during spray treatment. (D) Negative control, (E) 57 µL cinnamon leaf oil and (F) 500 µL ethanol treated mango during fumigation treatment.
Physicochemical properties: Spray treatment of essential oils did not adversely affect the physicochemical properties of mango (Table 02) including total soluble solids (TSS), titratable acidity (TA), pH and firmness which were evaluated after induced ripening. The TSS values obtained for all essential oil and carbendazim treatments (i.e. 22.20 – 26.40 °Brix) were not significantly different (p > 0.05) from the negative control (i.e. 22.80 °Brix). However, the present results are not in accordance with Sefu et al., (2015) who reported that TSS values of mango sprayed with cinnamon leaf and ginger oils were significantly lower than untreated controls. Gunasekera et al., (2018) reported that application of cinnamon bark oil (i.e. 0.20 µL/mL) incorporated edible wax coating on Karthakolomban mango stored at 13.5 ± 2 ºC for 1 week has slightly reduced TSS than the control. Further, TA values of each treatment (i.e. 0.42 – 0.50%) did not show a significant difference (p > 0.05) with respect to the negative control (i.e. 0.48%). According to Sefu et al., (2015), TA level of cinnamon leaf and ginger oil sprayed mango was in the range of 0.24 – 0.30% after 10 days from the treatment and these values are comparatively lower in comparison to the current study. TA values of cinnamon bark oil treated Karthakolomban mango (i.e. 0.31%) and control (i.e. 0.71%) reported by Gunasekera et al., (2018) are also lower when compared to the present results. pH values of all treatments (i.e. 3.86 – 4.18) were found to be significantly different from the negative control (i.e. 3.52) at p < 0.05. pH values of essential oil treated mango and control reported by Sefu et al., (2015) were within the range of 3.3 – 4.3 indicating the compatibility of results with the present research. According to Gunasekera et al., (2018), pH of Karthakolomban mango treated with cinnamon bark oil was 3.47 and it was relatively lower than the present values. Firmness of the clove oil treated mango (i.e. 0.39 kg cm⁻²) exhibited a significant difference compared to the negative control (i.e. 0.49 kg cm⁻²), but not the other treatments (i.e. 0.44 – 0.54 kg cm⁻²) (p < 0.05). According to Sefu et al., (2015), firmness levels of mango sprayed with cinnamon leaf oil at different concentrations showed a significant difference when compared to control and this result is not in agreement with present research. However, present findings are in agreement with Gunasekera et al., (2018) who reported that firmness of cinnamon bark oil treated Karthakolomban mango did not display a significant difference in comparison with the control. As a whole, mango sprayed with essential oils and carbendazim did not show any significant difference in its physicochemical parameters when compared to the negative control. Slight changes in certain physicochemical properties could be observed probably due to the variations in postharvest behavior of fruits and slight maturity differences (Anthony et al., 2003).

Table 02: Physicochemical properties of Karthakolomban mango subjected to spray treatment, stored at 12 – 14 ºC for 8 days and after induced ripening.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TSS¹ (ºBrix)</th>
<th>TA² (% citric acid)</th>
<th>pH</th>
<th>Firmness (kg cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>22.20 ± 1.59</td>
<td>0.50 ± 0.03</td>
<td>3.87 ± 0.04</td>
<td>0.49 ± 0.02</td>
</tr>
<tr>
<td>T2</td>
<td>25.20 ± 1.32</td>
<td>0.44 ± 0.01</td>
<td>3.86 ± 0.03</td>
<td>0.39 ± 0.02</td>
</tr>
<tr>
<td>T3</td>
<td>25.35 ± 1.01</td>
<td>0.42 ± 0.01</td>
<td>3.90 ± 0.02</td>
<td>0.44 ± 0.02</td>
</tr>
<tr>
<td>T4</td>
<td>26.40 ± 1.12</td>
<td>0.50 ± 0.03</td>
<td>4.02 ± 0.05</td>
<td>0.53 ± 0.02</td>
</tr>
<tr>
<td>T5</td>
<td>22.80 ± 1.32</td>
<td>0.48 ± 0.03</td>
<td>3.52 ± 0.03</td>
<td>0.49 ± 0.02</td>
</tr>
<tr>
<td>T6</td>
<td>24.90 ± 1.03</td>
<td>0.44 ± 0.03</td>
<td>4.18 ± 0.11</td>
<td>0.54 ± 0.02</td>
</tr>
</tbody>
</table>

¹Total Soluble Solids; ²Titratable Acidity; Each data point represents the mean of ten replicates ± standard error. Means sharing a common letter(s) within the same column are not significantly different by Tukey’s pair-wise comparison test at p < 0.05.

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**Peel colour:** The peel colour of mango subjected to spray treatment of essential oils and the respective positive and negative controls, were at the stages of fully green (index value = 1) or colour break (index value = 2) at the end of the 8-day storage period. After induced ripening, fruits attained the stages of more yellow than green (index value = 4) or fully yellow (index value = 5) within 1 – 2 days according to the peel colour index developed by Kodituwakku (2018). Mean peel colour of ripened mango of different treatments and controls were within the range of 4.5 – 4.8 and there was no significant difference (p > 0.05) of peel colour among the treatments, when compared to the control. This result is not in conformity with Karunanayake et al., (2018), who reported that basil and cardamom oil treatments (i.e. 0.70 µL/mL) have significantly altered the peel colour of Karthakolomban mango. Nisansala et al., (2016) reported that preharvest spray treatment of KCl (i.e. 1 – 4 gL⁻¹) on mango (cv. TomEJC) has not significantly affected peel colour. Even though treatment methods followed by Nisansala et al., (2016) were different, findings with regard to peel colour of mango are in accordance with the present study.

**Sensory properties:** Sensory attributes of mango sprayed with essential oils and carbendazim were not significantly different (p > 0.05) from the control. All scores were better than five, except for the flavour of basil oil treated mango. Sensory panelists, however, preferred the carbendazim treated mango than the ones treated with essential oils, which is evident by highest scores for all sensory parameters, except for texture (Table 03). However, the differences observed between treatments for all sensory properties seemed negligible.

According to Karunanayake et al., (2018), sensory properties of Karthakolomban mango treated with essential oils were not significantly affected when compared to the control and this observation is in conformity with the current study. Sarananda et al., (2004) reported that hot Ethral dip treatment at different temperatures (i.e. 26 – 52 °C) did not affect the flavour and overall acceptability of Karthakolomban mango. However, the result is consistent with present study irrespective of the treatments applied.

**Fumigation treatment for mango with essential oils**

**Pathological properties:** SER severity of mango fumigated with different essential oils was significantly different with respect to the negative control treated with distilled water (p < 0.05).
However, there was no significant difference in % SER of ethanol fumigated mango (i.e. the positive control) compared to the negative control. The highest % SER was evident in the negative control (i.e. 19.5%), while the second highest % SER was observed in mango treated with 500 µL ethanol (i.e. 14.5%). Mango treated with basil (142 µL), clove (142 µL), cinnamon leaf (57 µL) and cinnamon bark (28 µL) oils displayed % SER values (i.e. 0.5 – 2.0%) significantly lower than the negative control (Fig.05). Even though ethanol treatment was taken as the positive control it did not control SER in mango, unlike other treatments (Fig.04D – F). This observation complies with in vitro application of ethanol in the disc volatilization bioassay, which did not inhibit the growth of SER associated fungi, completely (Ekanayake, 2017; Kodituwakku, 2018). Dubey et al., (2008) have found that the fumigation treatment of essential oil of Amomum subulatum Roxb. leaves was capable of controlling SER of mango (cv. Dasheri) up to 8 days. According to Dubey et al., (2007), SER of the same mango variety was controlled by Eupatorium cannabinum Linn. oil fumigation for 7 days. Although mango cultivars and test oils are different, the duration of the shelf life of mango observed in these studies are in agreement with present study.

Physicochemical properties: Among all fumigation treatments, cinnamon bark oil treated mango exhibited relatively more desirable physicochemical properties than other treatments (Table 04). Those mangoes showed the highest TSS value (i.e. 35.25 °Brix) and therefore, cinnamon bark oil made mango sweeter. Samarawickrama et al., (2018) studied the fungicidal effect of hexanal incorporated into a composite packaging material made of banana fibre and polymers on extending the storage life of TomEJC mango. According to their results, TSS of mango packed with the composite material (i.e. 15.50 °Brix) followed by storage at 13.5 ± 2 °C for 1 week was not affected by the treatment. This is consistent with present study since most of the essential oil treatments have not significantly altered TSS of mango (i.e. 27.90 – 28.35 °Brix) except cinnamon bark oil when compared to the control. Cinnamon bark oil treatment of the current research is not in agreement with Tzortzakis (2007) who reported that TSS of strawberry stored at 13 °C for 6 days was not significantly affected by cinnamon bark oil fumigation. TA is an important measure of the organic acid content present in fruits which affect the palatability, pH of fruits is dependent on the total quantity and the strength of organic acids. Low organic acids make the fruits less acidic, hence increase the pH (Jayawickreme, 2002). Low TA (i.e. 0.36%) and high pH (i.e. 4.38) indicate that cinnamon bark oil treated mango was less acidic and more palatable than mango of other treatments. Cinnamon bark oil fumigation could be identified as the only treatment that showed a significant difference of TA when compared to the negative control (i.e. 0.45%) at p < 0.05. However, clove, cinnamon leaf and bark oil treatments demonstrated a significant difference in pH (i.e. 4.20 – 4.38) with respect to the ethanol treatment (i.e. 3.39) at p < 0.05. According to Samarawickrama et al., (2018), TA of hexanal treated TomEJC mango subjected to cold storage for 7 days was 0.83% and it was comparatively greater when compared to present results. However, pH of hexanal treated mango (i.e. 3.93) reported by Samarawickrama et al., (2018) was somewhat similar to present results. Further, Tzortzakis (2007) reported that when strawberry was fumigated with cinnamon bark oil, pH in the fruit was not affected. As fruits ripen, firmness is gradually reduced due to the enzymatic degradation of pectin, hemicellulose, polysaccharides and starch (Jayawickreme, 2002). Therefore, maintaining a higher firmness in ripe fruits could reduce their postharvest damages. Firmness of basil and cinnamon bark oil fumigated mango (i.e. 0.55 kg cm⁻²) was relatively greater than other treatments (i.e. 0.48 – 0.53 kg cm⁻²) which could contribute to longer shelf life. According to Samarawickrama et al., (2018), firmness of hexanal treated mango was not affected due to treatments and this observation is in agreement with the results of clove and cinnamon leaf oil fumigations.
Figure 05: SER disease severity of Karthakolomban mango subjected to fumigation treatment, stored at 12 – 14 ºC for 8 days and after induced ripening. T1: 142 µL basil oil, T2: 142 µL clove oil, T3: 57 µL cinnamon leaf oil, T4: 28 µL cinnamon bark oil, T5: negative control and T6: 500 µL ethanol. Each data point represents the mean of ten replicates. Means sharing a common letter(s) are not significantly different by Kruskal Wallis non-parametric test at p < 0.05.

Table 04: Physicochemical properties of Karthakolomban mango subjected to fumigation treatment, stored at 12 – 14 ºC for 8 days and after induced ripening.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>TSS¹ (ºBrix)</th>
<th>TA² (% citric acid)</th>
<th>pH</th>
<th>Firmness (kg cm⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>28.20± 1.09</td>
<td>0.51± ± 0.02</td>
<td>3.74± 0.37</td>
<td>0.55± 0.02</td>
</tr>
<tr>
<td>T2</td>
<td>28.35± 0.91</td>
<td>0.41± ± 0.01</td>
<td>4.33± 0.04</td>
<td>0.53± 0.02</td>
</tr>
<tr>
<td>T3</td>
<td>27.90± 0.98</td>
<td>0.43±± ± 0.01</td>
<td>4.20± 0.07</td>
<td>0.48± 0.01</td>
</tr>
<tr>
<td>T4</td>
<td>35.25± 1.33</td>
<td>0.36± ± 0.02</td>
<td>4.38± 0.07</td>
<td>0.55± 0.02</td>
</tr>
<tr>
<td>T5</td>
<td>29.40± 0.60</td>
<td>0.45±± ± 0.01</td>
<td>3.76± 0.08</td>
<td>0.48± 0.01</td>
</tr>
<tr>
<td>T6</td>
<td>30.00± 0.45</td>
<td>0.49±± ± 0.02</td>
<td>3.39± 0.06</td>
<td>0.52±± ± 0.01</td>
</tr>
</tbody>
</table>

T1: 142 µL basil oil, T2: 142 µL clove oil, T3: 57 µL cinnamon leaf oil, T4: 28 µL cinnamon bark oil, T5: negative control and T6: 500 µL ethanol.
¹Total Soluble Solids; ²Titratable Acidity;
Each data point represents the mean of ten replicates ± standard error.
Means sharing a common letter(s) within the same column are not significantly different by Tukey’s pair-wise comparison test at p < 0.05.

Peel colour: Peel colour of mango subjected to fumigation treatment was at the fully green (index value = 1) stage after 8-day storage period at 12 – 14 ºC. Induced ripening of mango changed the fully green peel colour to the stages of more yellow than green (index value = 4) and fully yellow (index value = 5). Mean peel colour of clove oil treated mango was 4.90, while 4.70 was the peel colour observed in other treatments including the positive and negative controls. Peel colour of all treatments was not significantly different (p > 0.05) from the negative control. In conformity with present results, Anthony (2003) reported that fumigation treatment of basil oil has not significantly affected the peel colour of Embul banana before and after induced ripening. Further, Ahmed (2019) proposes that harvesting methods and fruit size may also affect the peel colour of mango.

Sensory properties: The sensory panelists ranked the sensory properties of cinnamon
bark oil treated mango at a value around six compared to the rest of the treatments. When considering the overall acceptability, cinnamon bark oil treated mango was more preferred by the taste panelists than other treatments. Flesh colour, aroma and texture of mango subjected to different fumigation treatments were not significantly different (p > 0.05) with respect to the negative control. Taste of basil, clove and cinnamon bark oil and ethanol fumigated mangoes were significantly different from the control (p < 0.05). Flavour of mango treated with basil oil, cinnamon bark oil and ethanol were significantly different when compared to the negative control (p < 0.05) (Table 05). Consumer taste preference of mango is often thought to be related to TSS. During ripening, starch in the fruit pulp is hydrolyzed into reducing and non-reducing sugars. Therefore, the increase in sugar subsidizes the sweetness of ripe mango (Jayawickreme, 2002). This explanation is essentially consistent with the findings of the present study. For both fumigation and spray treatments, mangoes with high TSS values have scored higher sensory rankings for taste and flavour. Basil and cinnamon bark oil treated mango showed a significant difference in terms of overall acceptability, with respect to the control (p < 0.05). Different treatment strategies with botanicals might not be the only cause that influence the sensory attributes of mango. According to Ahmed (2019), harvesting methods and fruit size could significantly affect the sensory properties and marketability of mango. According to Anthony (2003), sensory properties of banana treated with basil oil were not significantly different from untreated control and these observations are compatible with the results of the present study.

SER severity values of essential oil treated mango was less than 2% for all essential oils used as sprays or fumigants and was also significantly less than the negative controls. However, the spray treatment of cinnamon bark oil could be identified as the most effective, since it was capable of completely controlling SER of mango stored at 12 – 14 ºC for 8 days. Therefore, other treatments need to be further improved to achieve a total control of the disease. In addition, further improvements of treatments could be focused to the storage life of mango with delayed ripening, since ripening of most of the mango fruits stored at 12 – 14 ºC was initiated approximately 7 – 9 days later. It is also important to have mature, green and firm mangoes without any physiological disorders or disease symptoms to minimize the mechanical, pathological and other postharvest damages that could aggravate during transport local or long distance (Sarananda and Amarakoon, 1999).

Table 05: Sensory properties of Karthakolomban mango subjected to fumigation treatment, stored at 12 – 14 ºC for 8 days and after induced ripening.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Flesh colour</th>
<th>Aroma</th>
<th>Texture</th>
<th>Taste</th>
<th>Flavour</th>
<th>Overall acceptability</th>
</tr>
</thead>
<tbody>
<tr>
<td>T1</td>
<td>6.06 ± 0.23</td>
<td>6.13 ± 0.33</td>
<td>6.00 ± 0.27</td>
<td>5.00 ± 0.30</td>
<td>4.75 ± 0.35</td>
<td>5.56 ± 0.20</td>
</tr>
<tr>
<td>T2</td>
<td>7.13 ± 0.40</td>
<td>5.75 ± 0.28</td>
<td>6.00 ± 0.32</td>
<td>4.94 ± 0.32</td>
<td>4.44 ± 0.35</td>
<td>5.13b ± 0.22</td>
</tr>
<tr>
<td>T3</td>
<td>6.56 ± 0.40</td>
<td>5.56 ± 0.26</td>
<td>5.63 ± 0.35</td>
<td>4.25 ± 0.21</td>
<td>3.81 ± 0.21</td>
<td>4.69b ± 0.20</td>
</tr>
<tr>
<td>T4</td>
<td>6.69 ± 0.38</td>
<td>6.25 ± 0.34</td>
<td>5.69 ± 0.29</td>
<td>5.75 ± 0.32</td>
<td>5.19 ± 0.29</td>
<td>6.00 ± 0.27</td>
</tr>
<tr>
<td>T5</td>
<td>6.69 ± 0.34</td>
<td>6.00 ± 0.39</td>
<td>6.19 ± 0.31</td>
<td>4.44 ± 0.26</td>
<td>4.25b ± 0.30</td>
<td>4.75b ± 0.21</td>
</tr>
<tr>
<td>T6</td>
<td>6.13 ± 0.31</td>
<td>5.63 ± 0.33</td>
<td>6.00 ± 0.29</td>
<td>4.81 ± 0.23</td>
<td>4.69 ± 0.27</td>
<td>5.00b ± 0.26</td>
</tr>
</tbody>
</table>

T1: 142 µL basil oil, T2: 142 µL clove oil, T3: 57 µL cinnamon leaf oil, T4: 28 µL cinnamon bark oil, T5: negative control and T6: 500 µL ethanol.

Each data point represents the mean of twenty replicates ± standard error.

Means sharing a common letter(s) within the same column are not significantly different by Kruskal Wallis non-parametric test at p < 0.05.
Taking the pathological, physicochemical and sensory properties of mangoes subjected to essential oil treatments into consideration, cinnamon bark oil treatments could be developed for commercial application in order to introduce mango fruits to the market with a better postharvest quality. Cinnamaldehyde, which has been identified as the major component in cinnamon bark oil (Kodituwakku, 2018) has been confirmed to be an antifungal compound in many studies (Hong et al., 2015; Marei and Abdelgalei, 2018). Few other components including eugenol and caryophyllene were detected as minor compounds in smaller quantities in addition to cinnamaldehyde. Compounds like camphene, phellandrene and terpineol were present in cinnamon bark oil as trace components in very low quantities (Kodituwakku, 2018). These antimicrobial components present in essential oils exhibit a wide array of mechanisms at cellular level in inhibiting growth and multiplication of microbial cells (Zaker, 2016). Herath and Abeywickrama (2008) reported the leakage of cell contents of Colletotrichum musae and Fusarium proliferatum conidia treated with essential oils of Ocimum basilicum, Cymbopogon citratus, eugenol and citral. Different antifungal components present in a single oil, in different proportions, displaying different modes of action could act synergistically to inhibit the growth of target fungi (Anthony et al., 2004).

CONCLUSIONS

Based on the data gathered by pathogenicity assessment, L. theobromae, Pestalotiopsis sp., Phomopsis sp. and X. fejeensis are confirmed as fungal pathogens of SER in mango. Contribution of Phomopsis sp. towards the disease was less than others and this is the first report on identifying X. fejeensis as a potential SER pathogen of Karthakolomban mango in Sri Lanka. Spraying Karthakolomban mango with emulsions of basil, clove and cinnamon leaf oils effectively controlled SER disease up to a satisfactory level during the storage at 12 – 14 ºC. A spray treatment of 1.6 µL/mL cinnamon bark oil completely controlled the disease up to 8 days without any unfavorable influence on physicochemical and sensory properties of mango. Fumigation with basil, clove, cinnamon leaf and cinnamon bark oils efficiently controlled SER disease of Karthakolomban mango up to an acceptable level during the storage at 12 – 14 ºC for 8 days. Sensory panel had a slightly higher preference towards cinnamon bark oil fumigated mango. Since the current treatments are capable of controlling SER of Karthakolomban mango up to about 1 week, those eco-friendly treatment strategies could be recommended to be used on commercial scale during transport and storage of mango for local trade. Mango treated this way could be exported via air freight to destinations which could be reached within a period of 3 – 4 days.

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